

PROTEINS OF THE GENUS *LITHOPS* (AIZOACEAE): DEVELOPMENTAL AND COMPARATIVE STUDIES

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ABSTRACT

The protein constitutions of species of the genus *Lithops* were examined by acrylamide gel electrophoresis. Total protein patterns show a strong developmental dependence and little taxonomic utility. Individual isoenzyme patterns show no developmental dependence and appear to be species-specific and of systematic interest.

UITTREKSEL

PROTEÏNE VAN DIE GENUS *LITHOPS* (AIZOACEAE): ONTWIKKELING EN VERGELYKENDE STUDIES.

Die proteïen samestellings van spesies van die genus *Lithops* was ondersoek deur akrylamiedgel elektroforese. Totale proteïen patrone toon 'n sterk ontwikkelings afhanklikheid en min taksonomiese bruikbaarheid. Individuele isoensieme patrone toon geen ontwikkelings afhanklikheid nie en blyk om van spesies spesifieke en sistematiese belang te wees.

INTRODUCTION

The taxonomy of the genus *Lithops* has historically been based primarily on morphological characters (Jacobsen, 1960). Recently studies of the microscopic anatomy of members of this genus have been initiated to determine if additional systematic insights might be gained (Dugdale, 1966; 1968). In numerous taxa, however, morphological and anatomical data have proven insufficient to resolve adequately some systematic questions which arise. Recently comparative studies of chemical constituents, both micromolecular and macromolecular, of closely related species have provided additional information concerning systematic relationships among various plant taxa (Alston, 1967; Turner, 1969). One species of *Lithops* (*L. kuibisensis*) has been reported to contain flower pigments in the class known as betacyanins (Wohlpert and Mabry, 1968). This chemical evidence supports the argument that the family Aizoaceae, of which *Lithops* is a member, is properly placed in the order Centrospermae along with the other plant families to which betacyanin pigments are unique. The present work reports chemical data on a different class of chemical compounds, the proteins. When the total extractable protein from a plant sample is fractionated by zone electrophoresis on acrylamide gels and appropriately stained, a pattern of bands is observed which has been found in some plant taxa to be species-

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specific and of diagnostic utility (Garber, 1965; Hart and Bhatia, 1967). To determine if such a situation obtains among various *Lithops* species, the present study was begun.

MATERIALS AND METHODS

Living plants and seeds were obtained from and identified by Mr. Chester Dugdale of Farleigh-Dickenson University, who had collected specimens in the field. Proteins were extracted from individual, mature plants by finely dicing the leaf material and grinding it with no further addition of water in a TenBroeck homogenizer. Natural tissue succulence provided adequate solvent for grinding. Sucrose was added to the homogenate to approximate a 20% solution and the homogenate was centrifuged (5 minutes at 1000xg) to remove cellular debris. The opalescent supernatant was layered directly as the electrophoretic sample. The small size of young seedlings necessitated pooling from 6 to 15 seedlings (depending on size and age) followed by grinding and preparation as above. Electrophoresis was carried out by standard methods of Ornstein and Davis (1962) at 3 milliamps per gel tube. Total protein was stained using Coomassie Blue (Chrambach, *et al.*, 1967). Enzyme specific assays were performed by the following methods: tyrosinase (polyphenol oxidase) (Davenport, 1964), alpha-esterase (Markert and Moller, 1959), lactate dehydrogenase (LDH) (Laycock *et al.*, 1965) and indophenol oxidase (Davenport, 1964).

RESULTS AND DISCUSSION

Developmental Studies

Current ontogenetic theory proposes that the process of differentiation is controlled by the selective synthesis of particular enzymatic proteins at different stages of development. It would be reasonable to expect that the protein banding pattern exhibited would be a function of the developmental stage of the organism. This has been observed in maize (Hamill and Brewbaker, 1969).

When *Lithops* seedlings from a single seeding of several species were examined for total protein pattern at intervals of several weeks, differences in the pattern could be detected with increasing age. Figures 1 and 2 show developmental changes in total protein pattern and compare seedling pattern with the pattern observed from seed material or mature plants where these were available. Marked developmental changes occur and, in general, the number of detectable bands tends to decrease with increasing age. It should be noted that this presents a serious problem in utilizing band pattern comparisons as a comparative taxonomic character since careful precautions would be required to insure that all taxa compared are at similar stages in development.

L. bella

L. salicola

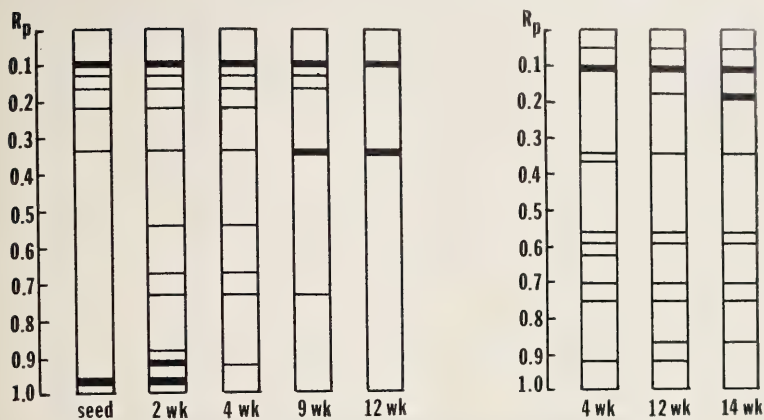


FIG. 1. Developmental changes in total protein in *Lithops bella* and *L. salicola*.

L. karasmontana

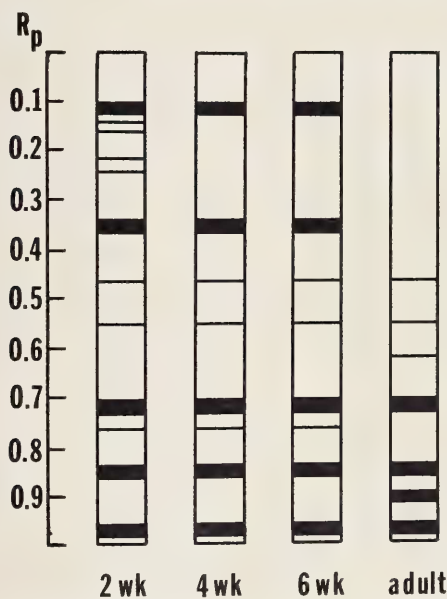


FIG. 2. Developmental changes in total protein in *Lithops karasmontana*.

In contrast with the total protein pattern, the banding pattern for specific enzymes showed no developmental dependence. The band pattern was identical from its earliest detection in young seedlings, through their subsequent development. This is a reflection of the fact that the particular enzymes examined are not those responsible for controlling differentiation in the developing seedling. Indeed, it would be highly fortuitous to find such an enzyme system in a survey such as is described herein.

Comparative Studies

The marked dependence of protein band pattern upon developmental stage prevents using it as a convenient taxonomic character. The results observed when enzyme-specific stains are used are in sharp contrast to the total protein

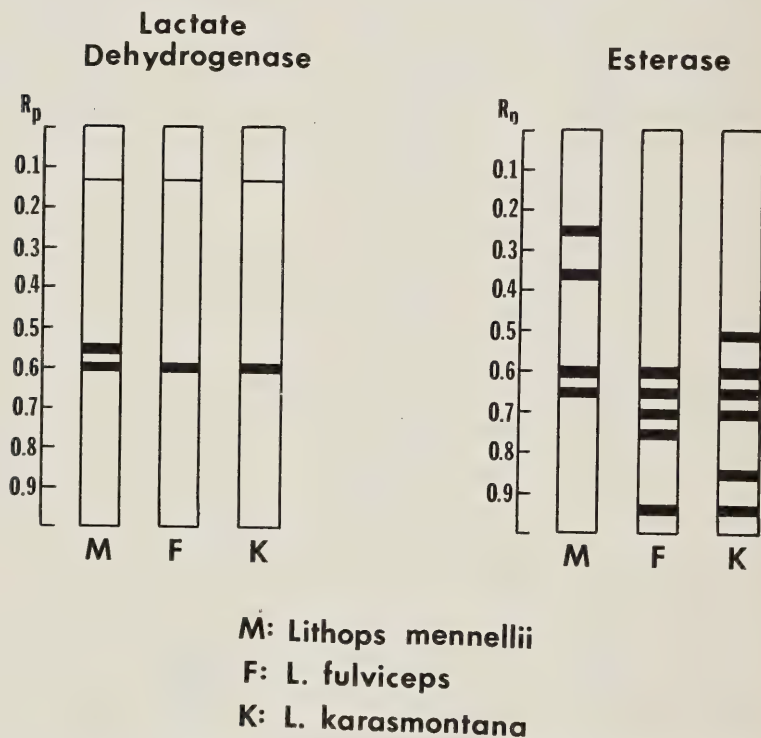


FIG. 3. Lactate dehydrogenase and alpha-esterase isoenzyme patterns of three *Lithops* species.

observations. Species-specific, potentially useful, isoenzyme banding patterns in seedlings are observed as illustrated in Figures 3 and 4. Efforts to stain for enzymes in adult plants were unsuccessful. This is presumably due to the fact that the metabolism of the adult plant is relatively dormant as compared with the actively growing seedling and the enzymes are present only in very small quantities. Polymorphism with respect to isoenzyme pattern has recently been observed in natural populations and cultivars of higher plants (Schwartz and Endo, 1966; Scogin, 1969; Wall, 1968). Such individual variations could not be observed in the present study because of the necessity of pooling numerous seedlings to provide an adequate sample for analysis.

The preliminary systematic conclusions inferred from comparisons of isoenzyme band patterns of several *Lithops* species contradict the currently accepted taxonomic positions of the species based on morphology (viz., *L. mennellii*, *L. fulviceps* and *L. karasmontana*). *L. fulviceps* and *L. mennellii* are placed together in the subgenus *Xantholithops* (Schwantes, 1951) on the basis of seedling morphology and flower colour, while *L. karasmontana* is placed in the second subgenus *Leucolithops*. Comparison of the LDH and esterase enzyme banding patterns reveal strong similarities (i.e., several bands with identical

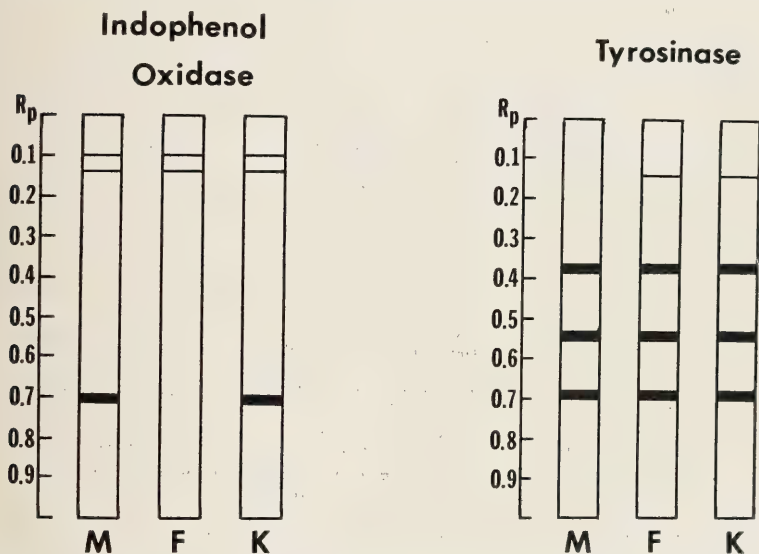


FIG. 4. Indophenol oxidase and tyrosinase isoenzyme patterns of several *Lithops* species. See Fig. 3 for species legend.

electrophoretic mobilities) between the patterns of *L. fulviceps* and *L. karas-montana* suggesting that they are more closely allied to each other than either is to *L. mennellii*. This conclusion is based on the assumption that bands with identical electrophoretic mobilities represent proteins with identical amino acid sequences (or, at least, the same net charge) and are therefore potentially homologous in their evolutionary derivation. These assumptions are not without weaknesses, as has been pointed out by Shaw (1965). An anomolous situation is noted in the indophenol oxidase patterns in that *L. mennellii* and *L. karas-montana* appear the most closely related pair. These results are preliminary and certainly do not form any basis for taxonomic revisions at the present stage, but they do suggest that a more extensive investigation into the utility of the chemical constituents as taxonomic characters in the genus is warranted. This study is being expanded to other species in an effort to detect systematic patterns among the various *Lithops* species and to determine the extent of intra-specific genetic variation as reflected by isoenzyme pattern polymorphism.

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